High-Fructose Diet Preserves β-Cell Mass and Prevents Diabetes in Nonobese Diabetic Mice: A Potential Role for Increased Insulin Receptor Substrate-2 Expression

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We demonstrate that a high-fructose diet reduces the incidence of diabetes in nonobese diabetic (NOD) mice (31.2% *v* 57.1% on regular chow (RC); *P* = .009). In a second cohort of mice, we evaluated potential mechanisms for the protective effect of the high-fructose (HF) diet and whether the metabolic changes are strain-specific. Sixty NOD and 60 Balb/c mice were randomized at weaning into HF- and RC-fed groups (30 mice each) and followed for 28 weeks. Glucose tolerance testing demonstrated improved glucose tolerance in HF diet groups (*P* = .001 in Balb/c; *P* = .04 in NOD mice at 6 months). β-cell mass was preserved in NOD mice on the HF diet, but remained unchanged in Balb/c mice. In NOD mice, hepatic insulin receptor substrate (IRS)-2 protein expression increased by 2-fold (*P* = .01 for 2 *v* 6 months) in HF-fed mice and was 53% ± 15% higher (*P* = .01) in the HF diet versus RC groups at 6 months of age. IRS-2 expression was also increased in skeletal muscle of NOD mice and in both liver and muscle of Balb/c mice. Our data suggest that a HF diet improves glucose tolerance in both NOD and Balb/c mice. The improved glucose tolerance may be related to increased IRS-2 expression and, in NOD mice, preservation of β-cell mass.

NONOBESE DIABETIC/LT (NOD) mice have been widely used as an animal model for the development of autoimmune (type 1) diabetes,[1,2] since 60% to 85% of NOD mice develop diabetes by the age of 30 weeks.[3] Interestingly, many studies suggest that autoimmune destruction of pancreatic beta cells in NOD mice can be both initiated and inhibited by dietary interventions. For example, the hypoallergenic infant formula, Pregestimil (Mead Johnson, Zeeland, MI) containing casein hydrolysate in place of protein, completely prevents diabetes in NOD mice,[4] whereas brewer's yeast or an undetermined chloroform-methanol-soluble substance(s) in natural-ingredient chow enhances the development of diabetes in these genetically susceptible mice.[3]

A diet enriched in fructose leads to the development of insulin resistance and hyperinsulinemia in normal, as well as in diabetes-prone rats.[5,6] Thus, in our studies of type 1 diabetes prevention, we assigned a group of NOD mice to a high fructose (HF) (60% fructose) diet, expecting the development of insulin resistance and an increase in diabetes incidence. To our surprise, however, by 40 weeks of age, the diabetes incidence was decreased (31.2% *v* 57.1%, *P* = .009) by the HF diet. We further characterized the diabetes-protective effect of a fructose-enriched diet in a new cohort of NOD mice and determined whether these metabolic changes also extended to Balb/c mice, a nondiabetes-prone strain.

RESEARCH DESIGN AND METHODS

Animals

All animal protocols were approved by the Institutional Animal Care and Use Committee of the Joslin Diabetes Center. Animals were housed in pathogen-free facilities and maintained on a 12-hour light/dark cycle.

Diet Composition

The HF diet was composed (wt/wt) of 60% fructose, 15% brewer's yeast, 12.2% casein, 4.5% lard, 3.2% cellulose, 5% Mineral Mix Rogers-Harper, and 1% vitamin mix TD40060 (Harlan, WI). The control diet was Purina Mouse Chow 9F (Purina Mills, Richmond, IN), containing wheat, yellow corn, soybean meal, wheat germ, fish meal, corn, gluten meal, brewer's yeast, animal fat, casein, and vitamin and mineral supplements similar to the HF diet (Table 1).

Protocol

For the initial phase of the study (phase I), we randomized 40 female NOD mice (Taconic Farms, Germantown, NY) at weaning (age 3 weeks) into HF- and RC-fed groups (20 mice each) and monitored them for 52 weeks for the development of diabetes. In the second cohort (phase II), 60 female NOD mice and 60 female Balb/c mice (Taconic) were randomized at age 3 weeks into HF- and RC-fed groups (30 mice each) and followed for 28 weeks.

Physiologic Testing

In both phase I and II, food intake, body weight, and random-fed glucose levels were monitored weekly. In phase I, fasting glucose and insulin levels were also measured at 2 months of age. The diagnosis of diabetes mellitus in NOD mice was based on polydipsia, weight loss, and persistent hyperglycemia of greater than 240 mg/dL, detected on at least 2 occasions using the Glucometer Elite (Bayer, Tarrytown, NY). Once an animal was diagnosed with diabetes, it was excluded from further analyses, including physiologic or tissue analyses.

In phase II, intraperitoneal glucose tolerance tests (GTT) and insulin tolerance tests (ITT) were performed at 2, 4, and 6 months of age on a cohort of randomly selected mice (n = 12 for HF and RC groups). In the NOD cohort, by age 6 months, 7 in the RC and 11 in the HF diet were available for the studies, as the others had developed diabetes. Numbers in the Balb/c cohort did not vary during the course of the study, as there were no deaths, and none developed diabetes. Intraperitoneal glucose tolerance testing was performed after an overnight fast; awake mice were injected with a 10% glucose solution (1.5 g/kg body weight) at time = 0, with blood obtained from the tail vein at 0, 15, 30, 60, and 120 minutes after injection for glucose measurement. For

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Table 1. Diet Composition

	HF Diet	Purine Mouse Chow 9F
Calories	3.82 kcal/g	3.75 kcal/g
Protein (wt/wt)	19.0%	20.5%
Fat (wt/wt)	12.5%	9.0%
Carbohydrate (wt/wt)*	65.4%	53.0%
Fructose	60.0%	0.02%
Glucose	<0.5%	0.02%
Sucrose		0.43%
Starch		38.5%

*Data from manufacturers (nonquantitated substances are fiber, ash, minerals, and moisture.

insulin tolerance testing, mice were injected with insulin (Humulin R [Eli Lilly, Centreville, VA] 0.5 U/kg body weight) at time = 0, followed by sampling for glucose at 15, 30, and 60 minutes. GTT and ITT were performed 1 week apart in all mice. Random-fed triglyceride levels were measured at 1, 3, and 5 months using the Triglyceride Gpo Kit (Synchron CX Systems, Beckman, Fullerton, CA).

Analysis of Insulin Signaling and Protein Expression

In phase II, 6 NOD and 6 Balb/c nondiabetic mice in each of the RC and HF groups were randomly selected at age 2, 4, and 6 months for analysis of insulin signaling and expression of insulin signaling proteins. Following an overnight fast, animals were anesthetized with sodium pentobarbital (50 mg/kg body weight, intraperitoneal). The abdominal cavity was opened, and 5 U of insulin (Humulin R) was injected into the inferior vena cava; liver and hind limb muscle tissues were removed and immediately frozen in liquid nitrogen at t = 1 and 2.5 minutes following insulin injection, respectively. Frozen liver and muscle tissue samples were subsequently homogenized using a Polytron (Brinkmann Instruments, Westbury, NY) and processed for immunoprecipitation and Western blotting as previously described.[7] Antibodies used for both immunoprecipitation and immunoblotting included anti–IRS-1 C-terminus,[7] anti–IRS-2 (generous gift of Morris White, Joslin Diabetes Center, Boston, MA),[8] anti-pY (4G10), or anti-insulin receptor C-terminus antibodies.[7] Proteins were detected using ^{125}I-protein A and quantified with the Molecular Dynamics Phosphorlmager and ImageQuant software (Sunnyvale, CA).

Histopathology

In phase II, pancreas was removed from anesthetized animals (4 nondiabetic mice/dietary group) at age 4 months. Pancreata were weighed, oriented in tissue cassettes, fixed in Bouin's solution, and embedded in paraffin. Sections (5/mouse, 5 to 7 μm) were immunostained using guinea pig antibodies against porcine insulin[9] and counterstained with hematoxylin. The β-cell mass was determined by point counting morphometry of insulin-positive cells as described.[10,11] Intersections with a 90-point grid were counted at 420 × final magnification; the whole tissue was covered without overlap. The relative volume of the β cell was calculated from the number of intersections over β cells divided by the total number of intersections over other pancreatic cells. At least 150 fields were counted over tissue in each animal. The same sections were used for grading insulitis in the NOD mice groups. The insulitis scorings were performed blindly by 2 independent observers using a semiquantitative scale from 0 to 4: 0, intact islet with no mononuclear cell infiltration; 1, focal peri-islet mononuclear cell infiltration; 2, more extensive peri-islet infitrates with lymphocytes less than one third of the islet area; 3, intraislet infiltrates up to one half of the islet area; 4, extensive intraislet infiltrates involving more than half of the islet area.[12] The islet cell regeneration was assessed by comparing the number of tiny islets (2 to 5 insulin–positive-stained cells) to the total number of islets and was expressed as a percentage of total islets.

Statistical Analysis

The values are expressed as mean ± SE. The Kaplan-Meier method was used for lifetable analysis, and Wilcoxon χ^2 statistics were applied for comparison of lifetable data. The diabetes frequencies are lifetable projections. A 2-tailed Fisher exact test was used to calculate P values for dichotomous variables. Analysis of variance (ANOVA) model was used to compare repeated measurements. The SAS System 6.12 (SAS Institute, Boston, MA) for Windows software was used for the calculations. Differences were considered significant when $P < .05$.

RESULTS

A HF Diet Decreases the Incidence of Diabetes in NOD Mice

Because HF diets have been shown to produce insulin resistance in rats, we treated a group of NOD mice (model of autoimmune or type 1 diabetes) with a 60% fructose diet, expecting an increase in diabetes incidence. To our surprise, the HF diet reduced the diabetes incidence. Lifetable analysis of NOD mice in phase I of the study shows that the HF diet reduces the incidence of diabetes by 40 weeks of age: 31.2% in the fructose-fed group compared with 57.1% in the mice on RC ($P < .01$) (Fig 1).

To evaluate potential mechanisms for the protective effect of a HF diet on diabetes incidence, we treated a new cohort of NOD mice (phase II) and analyzed pancreatic morphology, glucose and insulin tolerance, and other metabolic indices.

HF Diet Reduces Insulitis, Preserves β-Cell Mass, and Increases Small Islet Number in NOD Mice

Because the pathophysiology of diabetes in NOD mice is characterized by autoimmune insulitis and progressive loss of β-cell mass, we evaluated the magnitude of insulitis and β-cell mass in pancreata prepared from nondiabetic NOD mice from the HF and RC groups. At 4 months of age, the pancreatic insulitis score was significantly lower in the HF mice (1.09 ± 0.17) compared with the RC group (2.67 ± 0.27; $P = .004$).

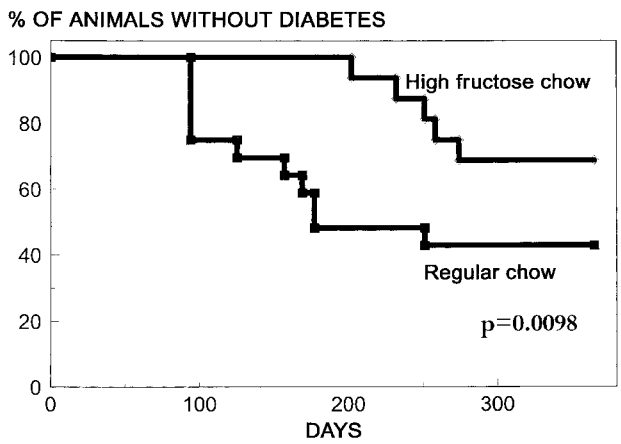

Fig 1. Lifetable analysis of NOD mice (study phase I). Diabetes incidence at 40 weeks of age was 31.2% in the fructose-fed group compared with 57.1% in the mice on RC ($P = .0098$).

Table 2. β-Cell Mass and Insulitis Scores at 4 Months of Age in NOD Mice

	β-Cell Mass (mg)	No. of tiny islets (2-5 cells)	Insulitis Score (including all islets)	Insulitis Score (excluding tiny islets)
HF	1.96 ± 0.4	77 (53.8%)	1.1 ± 0.2	2.2 ± 0.5
RC	0.56 ± 0.2	11 (24.4%)	2.7 ± 0.3	2.6 ± 0.7
Significance	$P = .05$	$P = .0008$	$P = .0004$	P = NS

NOTE. The β-cell mass was quantified by point-counting morphometrics of anti-insulin antibody- and hematoxylin-stained paraffin sections. Tiny cells were defined as 2 to 5 cell-clusters stained positive for anti-insulin antibodies and expressed in absolute number and as a percentage of total number of islets counted. Slides were graded for insulitis from 0 to 4 (4 mice/dietary group).

Abbreviations: HF, high fructose; RC, regular chow; NS, not significant.

This was due to the fact that intraislet infiltrates causing extensive insulitis (grades 3 and 4) were significantly fewer in the fructose-fed group than in the controls (40 of 143 HF *v* 22 of 45 RC; expressed as percentage of total islets counted, 27.9% HF group *v* 48.8% RC group, $P = .008$). We observed a large number of tiny islets (2- to 5-cell clusters stained positive for anti-insulin antibodies only and suggestive of regenerated islets[13]) in the HF-fed group. When we excluded the tiny islets from the analysis, the number of islets showing any signs of insulitis (grade 1 to 4) were similar in both dietary groups (44 of 66; 66.6% HF group *v* 26 of 34; 76.4% RC, P = not significant [NS]; insulitis score, 2.16 ± 0.47 in HF *v* 2.56 ± 0.65 in RC groups P = NS) (Table 2). The number of islets with extensive insulitis (grade 3 to 4) was also similar (32 of 66; 48.5% for HF group *v* 20 of 34; 58.8% for RC group; P = NS).

Pancreatic β-cell mass was higher in the HF-fed mice (1.96 ± 0.43 and 0.56 ± 0.24 mg in the HF and RC groups, respectively, $P = .05$). In addition, the number of tiny islets (2 to 5 β cells) was significantly higher in the fructose diet group compared with the controls (77 of 143 islets, 53.8%, *v* 11 of 45 islets, 24.4% respectively; $P = .0008$; Table 2).

To determine if these effects of a fructose-enriched diet extended to a nondiabetes-prone strain of mouse, we evaluated β-cell mass in Balb/c mice treated with HF and RC diets (n = 30/group). In contrast to the NOD mice, β-cell mass was similar in the HF and control groups (2.41 ± 0.35 HF *v* 1.75 ± 0.19 mg RC, P = NS). In addition, there was no significant difference in the number of tiny islets in the Balb/c mice (151 of 340 islets, 44.4%, *v* 182 of 382 islets, 47.6% respectively; P = NS).

HF Diet Improves Glucose Tolerance in Both NOD and Balb/c Mice

To assess the potential contribution of alterations in glucose tolerance and/or insulin sensitivity, which could contribute to the observed reduction in diabetes incidence in NOD mice, we performed glucose tolerance and insulin tolerance testing in nondiabetic phase II mice at 2, 4, and 6 months of age. In both NOD and Balb/c mice, glucose tolerance on HF and RC diets was similar at 2 months of age. By 4 months of age, glucose levels were significantly lower in the Balb/c HF group (ANOVA $P = .009$), and by 6 months of age, both NOD and Balb/c mice fed the HF diet demonstrated improved glucose tolerance, as reflected in both the initial and postglucose challenge glucose levels (Fig 2A and B) (ANOVA $P = .04$ and $P = .001$ in NOD and Balb/c groups, respectively). By contrast, there were no significant differences in responsiveness to the hypoglycemic effects of intraperitoneal insulin in either NOD or Balb/c mice (data not shown).

Because changes in weight and food consumption can influence glucose tolerance, we measured food intake and weight weekly. There was no significant difference in consumption of RC and fructose-rich chow in both NOD and Balb/c strains.

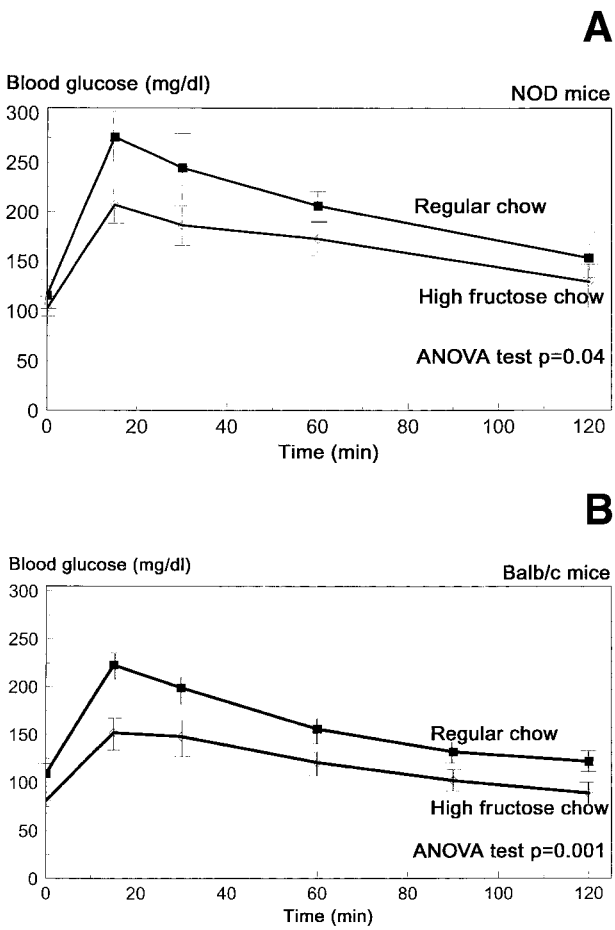

Fig 2. Glucose tolerance is improved on HF diet in NOD mice (A) and Balb/c mice (B) at age 6 months. Following an overnight fast, mice were injected with 1.5 g/kg glucose intraperitoneally; glucose values were determined at the indicated times. Glucose values were significantly lower in the HF group for both NOD (A, ANOVA $P = .04$ *v* RC) and Balb/c mice (B, ANOVA $P = .001$ *v* RC).

Table 3. Serum Insulin and Glucose Levels in Fed State at 1, 3, and 5 Months of Age in Both NOD and Balb/c Mice Groups on Different Dietary Regimes

	Insulin (μU/mL)		Glucose (mg/dL)		Insulin/Glucose Ratio	
	HF	RC	HF	RC	HF	RC
NOD mice						
1 month	110.8 ± 8.7*	64.4 ± 4.7	133.0 ± 2.4†	122.0 ± 3.8	0.87 ± 0.0‡	0.56 ± 0.0
3 month	90.6 ± 11.6§	90.7 ± 16.7	129.7 ± 5.2*	99.8 ± 2.9	0.71 ± 0.1§	0.91 ± 0.1
5 month	64.4 ± 28.8§	84.0 ± 23.4	155.5 ± 10.4§	135.7 ± 22.5	0.38 ± 0.1§	0.76 ± 0.2
Balb/c mice						
1 month	46.11 ± 2.0*	35.0 ± 0.9	123.5 ± 4.0§	129.7 ± 3.4	0.35 ± 0.0*	0.26 ± 0.0
3 month	34.0 ± 2.6*	13.2 ± 0.8	122.8 ± 3.5§	125.5 ± 2.6	0.28 ± 0.0*	0.10 ± 0.0
5 month	12.8 ± 1.1§	11.8 ± 0.5	124.1 ± 4.6§	117.0 ± 3.6	0.10 ± 0.0§	0.10 ± 0.0

NOTE. n = 8 mice/dietary group. Units for insulin/glucose ratio are μU/mL/mg/dL.
*P = .0001.
†P = .01.
‡P = .002.
§P = NS.

Similarly, body weight of the animals in each diet group did not differ significantly in NOD mice (data not shown). In Balb/c mice, weights were similar until age 24 to 28 weeks, when the weight of the HF diet mice increased (at 28 weeks: 22.9 ± 0.4 gm *v* 20.7 ± 0.4 gm for HF and RC diet, respectively; P = .002).

HF Diet Lowers Fasting Insulin and Glucose in NOD Mice and Lowers Fasting Triglycerides

To determine the potential role for fructose-mediated alterations in basal and postprandial insulin secretion, we measured insulin and glucose levels in both the fasting and fed state. Fasting insulin levels in NOD mice (phase I) were significantly reduced in the HF group (21.0 ± 2.7 μU/mL *v* 30.6 ± 3.7 μU/mL in HF *v* RC; P = .04). Fasting glucose levels were also lower in the HF group (60.6 ± 3.6 *v* 73.0 ± 4.6 mg/dL, respectively; P = .04) at 2 months of age. The insulin/glucose ratio remained unchanged (0.31 ± 0.1 *v* 0.38 ± 0.1 μU/mL/mg/dL in HF *v* RC; P = NS).

We measured fed insulin and glucose levels in both NOD and Balb/c mice in phase II. Fed insulin levels and insulin/glucose ratios were higher in both NOD and Balb/c fructose-fed mice at 1 month of age; by 3 months of age, these effects were seen only in the Balb/c strain and had dissipated by 5 months of age (Table 3). Fed-glucose levels were similar in both NOD and Balb/c mice on HF and RC diets. Fasting serum triglyceride levels in both NOD and Balb/c mice at 2, 4, and 6 months of age were significantly lower in the HF-fed groups (Table 4).

HF Diet Increases IRS-2 Protein Expression in Liver and Muscle

To evaluate potential changes in insulin sensitivity at a cellular level, which might explain the protective effect of the HF diet, we analyzed acute insulin signaling in liver and skeletal muscle following the injection of insulin in vivo in all nondiabetic mice in both HF and RC groups at 2, 4, and 6 months of age. Insulin significantly stimulated insulin receptor, IRS-1, and IRS-2 tyrosine phosphorylation, and association of IRS proteins with the p85 subunit of phosphatidylinositol 3-kinase in all mice. There were no significant differences in the magnitude of these responses in the HF versus RC groups in either NOD or Balb/c mice.

We also analyzed expression of insulin receptor, IRS-1, IRS-2, and p85 signaling proteins in liver and muscle from nondiabetic animals at ages 2, 4, and 6 months of age. There were no significant differences in insulin receptor, IRS-1, or p85 expression in the HF versus RC groups (data not shown). However, we noted a consistent and time-dependent increase in IRS-2 protein expression in liver of HF-fed mice. In NOD mice, IRS-2 expression in liver increased by 2-fold (P = .01 for 2 *v* 6 months) in HF mice and was 53% ± 15% higher in HF versus RC mice at 6 months of age (P = .01) (Fig 3). In Balb/c mice, hepatic IRS-2 expression was 1.6-fold and 2.2-fold higher in HF versus RC animals at 2 and 4 months of age (P < .01 and < .05, respectively). However, these differences were lost by 6 months of age (data not shown).

IRS-2 expression in hind limb skeletal muscle of NOD mice also increased over time in the HF diet group (2.5 fold greater in HF *v* RC at 4 months, P < .01); these changes remained significantly different from RC mice at 6 months (37% increase, P < .01) (Fig 4). Similar data were evident in Balb/c mice, with a 32% increase in IRS-2 expression in hind limb

Table 4. Fasting Serum Triglyceride Levels in NOD and Balb/c Mice on HF Diet and RC

	HF Diet (mg/dL)	RC (mg/dL)	Significance
NOD mice			
2 month	<100	130.4 ± 4.1	P = .0001
4 month	170.1 ± 6.3	232.6 ± 8.5	P = .0001
6 month	172.5 ± 13.9	367.7 ± 10.9	P = .0001
Balb/c mice			
2 month	131.1 ± 4.6	153.5 ± 6.1	P = .005
4 month	194.5 ± 3.1	257.4 ± 12.3	P = .0001
6 month	275 ± 2.4	417.5 ± 11.6	P = .0001

NOTE. In both strains, these values were significantly lower in the fructose-fed groups (n = 8 mice/dietary group).

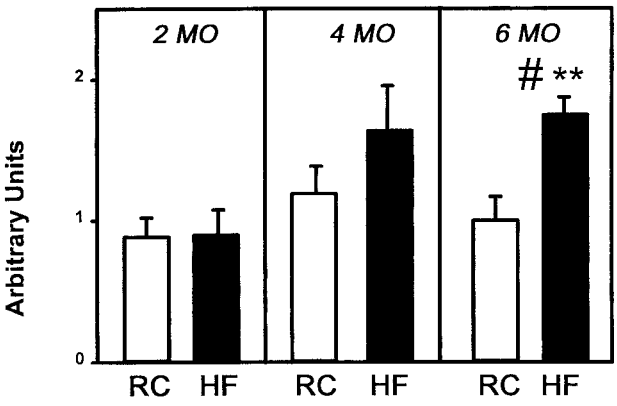

Fig 3. IRS-2 protein expression is increased in a time-dependent manner by HF feeding in liver of NOD mice. IRS-2 protein expression was quantitated from anti–IRS-2 immunoblots of anti–IRS-2 immunoprecipitates (separated in parallel on sodium dodecyl sulfate-polyacrylamide gel electrophoresis [SDS-PAGE] gels). Raw PhosphorImager intensities for all groups were normalized to the mean value for IRS-2 expression for all 6-month RC mice (chosen as a representative arbitrary reference due to greater sample number in this group). Data reflect 6 mice/dietary group. # Indicates *P* = .01 for HF at 6 months *v* 2 months; ** indicates *P* = .01 for HF *v* RC at 6 months.

muscle in the HF group as compared with the RC group at 6 months of age ($P = .02$) (data not shown).

DISCUSSION

Fructose is a monosaccharide with low-glycemic index, which is absorbed readily from the jejunum via GLUT5 facilitative fructose transporters. The liver extracts between 55% to 75% of portal fructose; the remaining fructose is extracted by other tissues, including kidney, adipose, and skeletal muscle via the GLUT5 transporter.[14,15] Fructose transport and metabolism are rapid and not regulated directly by insulin.[16] Fructose is phosphorylated by fructokinase; the subsequent action of aldolase B and triose kinase ultimately generates triosephosphates, which can enter glycolysis and be oxidized in the tricarboxylic acid cycle or can be converted to glucose or glycogen via gluconeogenesis. HF feeding increases intestinal and renal, but not skeletal muscle, GLUT5 expression.[17]

Fructose has several potential metabolic advantages over glucose in the dietary management of patients with type 1 diabetes and those at risk for type 2 diabetes. Postprandial increases in plasma glucose following consumption of fructose are lower than those produced by isocaloric amounts of dextrose or sucrose,[18] and fructose is not a potent stimulus for insulin secretion. One would predict that fructose might ameliorate the hyperinsulinemia and excessive food intake, which follows high glycemic index foods.[19] Moreover, the thermogenic response to fructose exceeds that of glucose and, in contrast to glucose, is not impaired in subjects with insulin resistance or diabetes.[18]

In hepatocytes or perfused liver, small amounts of fructose stimulate glucose uptake, glycolysis, and glycogen synthesis. In addition, intraportal delivery of fructose increases hepatic glucose uptake more than 3-fold in dogs, likely due to increased fructose-1-phosphate levels, release of glucokinase from its inhibitory protein, and thus activation of glucokinase enzymatic activity.[15,20] This results in net glycogen storgage, oxidation, and lactate production. In addition, other studies have demonstrated potentially beneficial effects of fructose on activation of hepatic glycogen synthase, largely due to allosteric activation by increased glucose-6-phosphate levels.[21]

Despite these potentially beneficial effects of fructose on carbohydrate metabolism, chronic administration of HF diets usually induces more severe insulin resistance, hyperlipidemia, and hypertension.[22] These effects may reflect the availability of fructose-derived 3-carbon precursors for enhanced fatty acid synthesis and triglyceride formation. In both normal and insulin-resistant, diabetes-prone rats, a diet enriched in fructose (>34% fructose) leads to the development of hyperglycemia, insulin resistance, and hyperinsulinemia.[5,6] Concern has been raised that these effects may be at least partly related to the ability of fructose to decrease bioavailability of copper.[23] In addition, fructose significantly increases the insulin content of β cells and, in combination with other sugars (mannose or glucose), results in a synergistic effect on insulin secretion and an additive effect on islet insulin content.[24] The pathophysiology of fructose-enriched feeding leading to increased insulin production and insulin resistance in rats remains unclear, but also may be linked to effects on hypertriglyceridemia and free fatty acid production, with secondary insulin resistance.

In humans, studies of the role of fructose in the metabolic control of diabetes have been largely short-term and confined to small numbers of subjects with type 2 diabetes. Some of these demonstrated improved glycemic control and insulin sensitivity (34% improvement in S_I after a 4-week diet containing 10% of total energy from fructose),[25] while others report a decline in insulin sensitivity and increased insulin concentrations with fructose supplementation (net hyperenergetic diet)[26,27] or no significant change.[28,29] Subjects with preexisting hypertriglyceridemia may be particularly susceptible to the effects of fructose on lipid metabolism.[30] Taken together, these data support

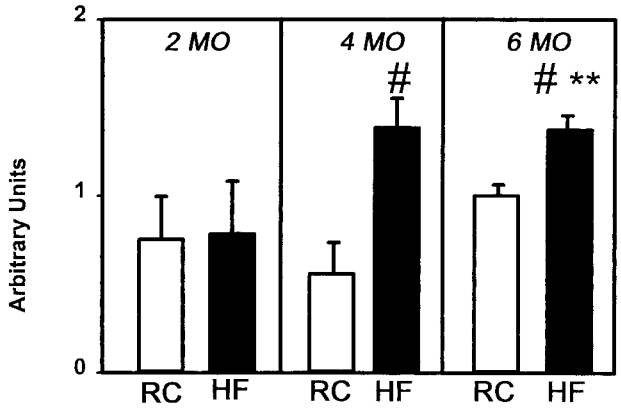

Fig 4. IRS-2 protein expression is increased in a time-dependent manner by HF feeding in muscle of NOD mice. IRS-2 protein expression was quantitated from anti–IRS-2 immunoblots of anti–IRS-2 immunoprecipitates and expressed in arbitrary PhosphorImager units, as described for Fig 3. Data reflect 6 mice/dietary group. # *P* < .01 for 6 months *v* 2 months for HF diet; ** indicates *P* < .01 for HF *v* RC at 6 months.

that for subjects with hyperinsulinemia and hypertriglyceridemia, fructose-rich diets may decrease insulin sensitivity and exacerbate hyperlipidemia.

Based on the above data, we expected that a HF diet would result in insulin resistance, hyperinsulinemia, β-cell hypersecretion, increased insulitis, and increased diabetes incidence in NOD mice used for our long-term studies of type 1 diabetes pathogenesis and pharmacologic prevention. To our surprise, however, as reported in the present study, a HF diet reduced the incidence of diabetes in NOD mice. In addition, the HF diet improves glucose tolerance in both NOD and Balb/c strains of mice. Potential mechanisms for these effects include alterations in peripheral or hepatic insulin sensitivity, improved non–insulin-mediated glucose disposal, β-cell rest, and increased β-cell mass.

The improvements in glucose tolerance observed in both NOD and Balb/c mice might be related to improvement in insulin sensitivity. We demonstrated no change in insulin sensitivity, as assessed by the ITT. However, this method is unlikely to be sensitive enough to detect more subtle changes in peripheral or hepatic insulin sensitivity. Thus, we cannot exclude with certainty the contribution of subtle improvements in hepatic insulin sensitivity to the observed effects of HF diet, but it is likely that these effects are small. Similarly, insulin-independent glucose disposal[31] may play an important role in the observed effects of the fructose-rich diet. Additional studies of both whole-body physiology and changes in hepatic metabolism, insulin sensitivity, and insulin-independent glucose disposal will be required to address these possibilities more fully.

Serum triglyceride levels were markedly decreased in the fructose-fed NOD and Balb/c mice. These data contrast markedly from those in rat studies, in which fructose diets increase serum triglyceride levels.[5,32] In humans, short-term studies demonstrate no change in serum triglycerides in subjects with normal fasting insulin levels[25,33,34]; the effect of HF diets on lipid metabolism in humans may be evident only in subjects with preexisting hyperinsulinemia and hypertriglyceridemia.[33] The mechanism by which the HF diet lowered triglyceride levels in our cohort of mice is unclear at present, but we postulate that improved hepatic insulin sensitivity and insulin secretion related to upregulation of IRS-2 protein expression may play a prominent role.

Our data more strongly support a role for β-cell rest and preservation of islet mass in the diabetes-protective effects of fructose in NOD mice. NOD mice on the HF diet had lower fasting glucose and insulin levels and improved glucose tolerance compared with mice on RC. This suggests the possibility that the HF diet reduced the metabolic demands on the β cells. This "β-cell rest" may lead to lower antigenicity of β cells and downregulation of the autoimmune response. Ex vivo cultures of mouse or rat islets at increasing glucose concentrations results in increased β-cell metabolic activity, increased expression of antigens IC2, A2B5,[35,36] and in monkeys, increased expression of glutamic acid decarboxylase (GAD65).[37] In addition, islets cultured in increasing glucose concentration are more susceptible to lymphokine-mediated destruction,[38] while "resting" β cells appear to be resistant to the effect of interleukin (IL)-1.[39] Thus, fructose, through its effects to decrease basal and postprandial glucose excursions, increase hepatic glycogen storage, and minimal effects on insulin secretion (in comparison to other carbohydrates) may decrease metabolic demands on β cells (β-cell "rest") and similarly reduce stimulus for autoimmune attack.

Pancreatic histologic analysis provides support for this potential mechanism of the diabetes-protective effect in NOD mice. The HF diet preserves β-cell mass, increases islet regeneration (increased numbers of tiny islets), and decreases the severity of insulitis in NOD mice. The reduction in insulitis score was largely due to an increased number of less inflamed tiny islets. Interestingly, the lack of change in β-cell mass in Balb/c mice may indicate that the HF diet enhances a compensatory regeneration triggered by extensive cell destruction, but does not accelerate the normal turnover of β cells.

The response of NOD and Balb/c mice to HF feeding demonstrates significant, and potentially important, differences between the strains. Notably, in RC-fed mice, the insulin/glucose ratio (expressed as μU/mL/mg/dL) is higher in NOD than in Balb/c mice (0.56 ± 0.04 *v* 0.26 ± 0.00 at 1 month; 0.91 ± 0.16 *v* 0.10 ± 0.00 at 3 months; 0.76 ± 0.24 *v* 0.10 ± 0.00 at 5 months of age; $P = .0001$, $P = .0002$, and $P = .02$, respectively; Table 3). In the HF-fed groups at 1 and 3 months of age, the insulin/glucose ratios are again higher in NOD mice, but at 5 months, these interstrain differences become insignificant (0.87 ± 0.08 *v* 0.35 ± 0.01; 0.71 ± 0.11 *v* 0.28 ± 0.02; and 0.38 ± 0.16 *v* 0.10 ± 0.01; $P = .0001$, $P = .001$, and $P =$ NS, respectively; Table 3). These data may suggest that the HF diet reduces relative insulin secretion in the NOD mouse (β-cell rest) closer to that of the less-susceptible Balb/c strain. Thus, we may speculate that the effects of fructose as a dietary and potential therapeutic manipulation may relate to the underlying genotype and metabolic phenotype of the β cell.

What are the potential mechanisms for these β-cell preserving effects? It is intriguing that the fructose-enriched diet increases expression of IRS-2 in liver and muscle over time. IRS-2 is a key insulin receptor substrate protein expressed in liver, pancreatic ductal cells, and β cells. IRS-2 is critical for both hepatic insulin sensitivity[40] and β-cell mass; mice lacking IRS-2 develop overt diabetes, which appears to result from both low β-cell mass and hepatic insulin resistance.[41] In addition, IRS-2 is required for insulin-like growth factor (IGF)-1 receptor-mediated β-cell development and survival and compensation for peripheral insulin resistance.[42] Thus, in both NOD and Balb/c mice, the increased expression of IRS-2 may improve hepatic insulin sensitivity or result in altered gene expression favoring glucose uptake and reduced gluconeogenesis. More importantly, the enhanced IRS-2 expression may be a marker for diet-induced alterations in IRS-2 epxression in the β cell. Thus, we postulate that the fructose-enriched diet enhances IRS-2 expression in β cells; enhanced IRS-2 may play a role in islet cell regeneration and preservation of β-cell mass despite autoimmune insulitis, leading to decreased gluconeogenesis and β-cell rest, islet preservation, and diabetes prevention. Additional experiments are in progress to address this hypothesis.

In conclusion, a HF diet improves glucose tolerance in both NOD and Balb/c mice, increases expression of IRS-2 in liver

and muscle, preserves β-cell mass, and reduces diabetes incidence and β-cell hyperactivity in the NOD mouse. Thus, our data support a role for fructose as a potential dietary tool in diabetes prevention. Further investigation of mechanisms for the regulation of IRS-2 expression by fructose-enriched diets and use of HF or low-glycemic diets for diabetes prevention in humans will be required to address these intriguing possibilities.

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